# Clonal dynamics of the memory B-cell repertoire in response to a PfRH5-based malaria vaccine candidate

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## Main objectives and findings

- The PfRH5 protein is essential for the pathogenesis of *Plasmodium falciparum* malaria. We used B-cell receptor IGH repertoire sequencing to study patient response to a PfRH5-based, adenovirus/MVA prime-boost vaccine candidate.
- Clonal expansion was observed in patient memory repertoires 140 days after vaccination. Individuals had disparate responses, with expansions of different clonotypes (clonotype = V-J gene + CDR3 length), and differing proportions of known anti-PfRH5 antibodies in their repertoires.
- Expanded clonotypes had a higher mutational frequency and were mostly IgA isotypes, suggesting activation of a mucosal response.

## Background

### No effective vaccine exists for malaria

- In 2015, malaria caused 429,000 deaths globally, of which 99% were attributed to *Plasmodium falciparum* (**Pf**), the most virulent malaria parasite species[1].
- Vaccine development efforts are plagued by the parasite's complex life cycle, redundancies in invasion pathways, and antigenic variability.
- The only licenced Pf vaccine to date is RTS,S/AS01. Its efficacy in Phase III clinical trials was 30-50%, waning rapidly over time[2].

### PfRH5 is an high-priority vaccine target

- Interaction of **PfRH5** (a Pf merozoite surface protein) and basigin (an erythrocyte receptor) is essential for erythrocyte invasion (Fig. 1).
- A PfRH5-based vaccine has been shown to be efficacious against infection by heterologous strains of Pf in non-human primate models.

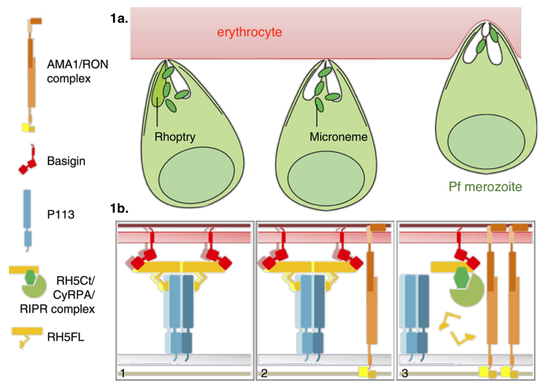


Figure 1: A model of erythrocyte invasion by a Pf merozoite (1a), with a closeup of the extracellular interactions involved (1b): 1. secretion of RH5 from the rhoptries and tethering by P113; 2. AMA1/RON complex initiates moving junction formation; 3. CyRPA and RIPR secretion from the micronemes and tether release, forming the invasion complex. *Figure source*[3].

### Using BCR Rep-seq to investigate immune response to vaccination

- B lymphocytes (B-cells) are part of the adaptive immune response. They produce immunoglobulins (Igs) in two forms: secreted antibodies and membrane-bound B-cell receptors (BCRs).
- Igs are comprised of heavy and light peptide chains. Antigen binding is largely mediated by the heavy chain, which is encoded by the **IGH locus**.
- During B-cell maturation, V(D)J-recombination (Fig. 2) contributes to the diversity required to recognize a wide range of antigens. The circulating B-cells in an individual (their **B-cell repertoire**) have an estimated IgH diversity of at least $10^6$ [4].

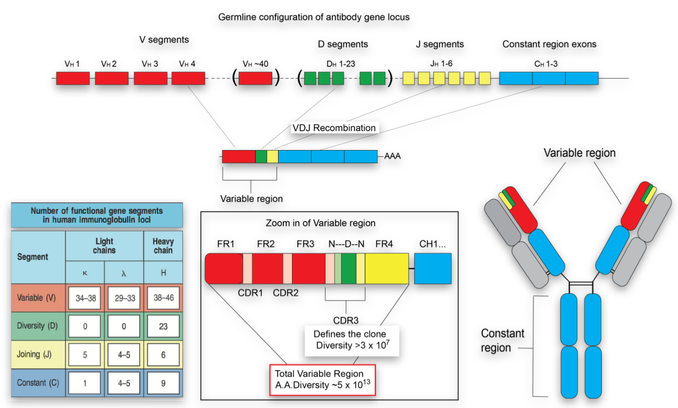


Figure 2: The process of V(D)J-recombination at the IGH locus during B-cell maturation in the bone marrow. Somatic recombination of gene segments and addition/deletion of nucleotides at the junctions (within the CDR3 region) generates BCR repertoire diversity. *Figure source*[5].

- Serum Ig responses are critical for clinical protection against Pf; passive immunity can be conferred by Ig transfer from hyperimmune to susceptible individuals.
- Activated B-cells can undergo clonal expansion, somatic hypermutation, and class switching. We used immune repertoire sequencing (**Rep-seq**) to characterize the BCR repertoire after vaccination and identify potential correlates of immune response.

## Methods and study design

### The IsoTyper pipeline for IgH RepSeq from total RNA

- Multiplex priming on the conserved IGH1 FR1 (5'primer set) and constant regions (3'primer set) captures full repertoire diversity, and allows identification of all Ig isotypes in a single reaction (Fig. 3).
- Unique molecular barcoding of RNAs during reverse transcription allows downstream algorithmic correction of PCR and sequencing errors.
- The IgH sequences are assembled using overlap between paired-end reads.

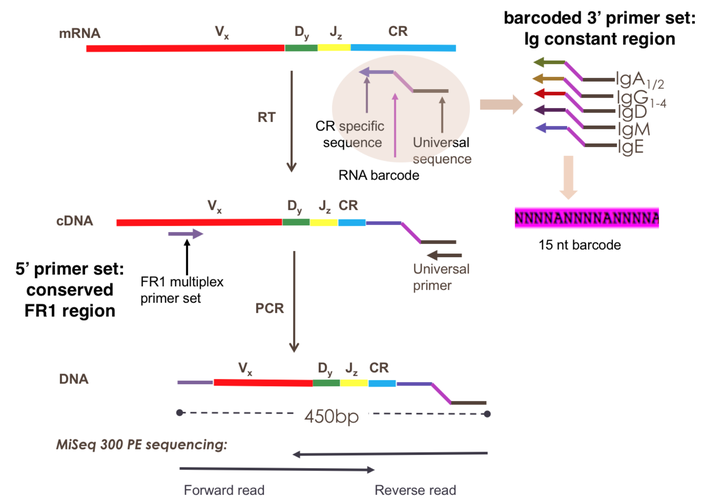


Figure 3: Targeted amplification of BCR IgH sequences from total RNA using the IsoTyper 3'MPLX method[6].

### Phase I clinical trials of a PfRH5-based vaccine candidate

- Three patients were vaccinated with an PfRH5-based adenovirus/DNA prime, followed by a PfRH5-based modified vaccinia Ankara (MVA) boost after 8 weeks. Peripheral blood samples were taken at four time points (Fig. 4).
- PBMCs were isolated (Ficoll-Paque method), then further sorted using fluorescence-activated cell sorting into $CD19^+CD27^+$ memory B-cell and $CD19^+CD27^+CD138^+$ plasmablast populations.

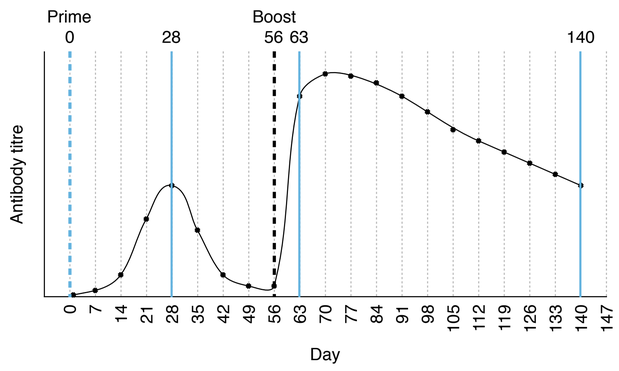


Figure 4: Hypothetical individual blood antibody titre during the PfRH5-based prime-boost vaccination schedule. Sampling time points are show in blue.

- Total RNA was isolated (QIAGEN RNeasy Plus), and B-cell IgH repertoires were sequenced using the IsoTyper pipeline. V gene segment, J gene segment and CDR3 region boundaries were assigned using IMGT/HighV-QUEST[7].

## Pilot analysis

### Dynamics of the memory BCR repertoire before and after vaccination

- Each B-cell **clone** produces Igs of a single IgH **clonotype**. We defined clonotype as the combination of *V gene segment, J gene segment, and CDR3 region amino acid length*.
- Comparing the memory repertoires of malaria-naive (day 0, pre-prime) and vaccinated individuals (day 140) allows identification of clonotypes that may contribute to long-term immunity.

### Clonal expansions occurred in response to vaccination

- The overall diversity of the memory repertoire decreases after vaccination in two out of three patients (Fig. 5) ...

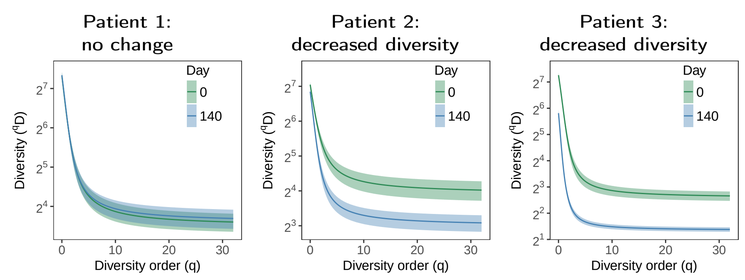


Figure 5: Hill diversity curves for the memory repertoire, before and after vaccination (95% CI computed using 200 bootstraps).

- ...due to clonal expansion of B-cells with certain clonotypes (Fig. 6).

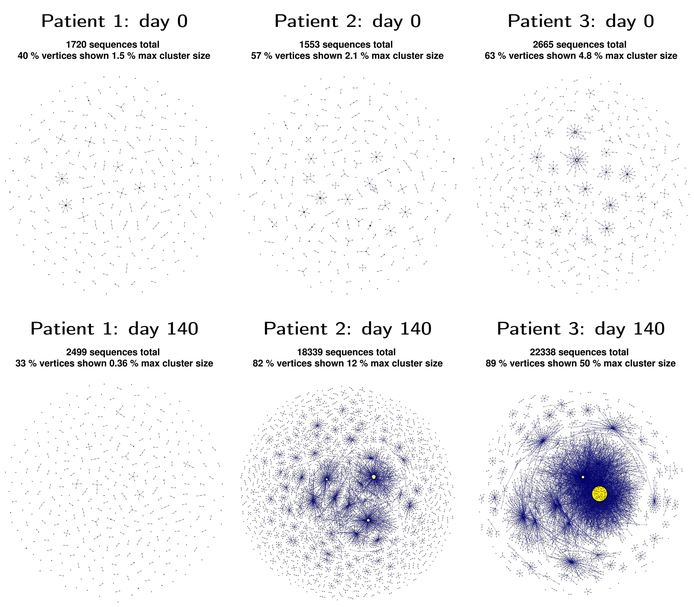


Figure 6: Network representation of BCR sequences. Nodes represent clones with unique antibody sequences. Node size indicates number of reads. Singleton clones are not shown. Edges connect sequences that differ by one base pair (substitution or indel).

### Comparison of expanded clonotypes with known anti-PfRH5 clonotypes

- The 22234 unique clones detected in the memory repertoires consisted of 1626 clonotypes, which were compared to the clonotypes of eighteen previously characterised monoclonal antibodies with known binding affinity to PfRH5.
- Twenty clonotypes were classified as expanded (with a significant increase in their repertoire proportions after vaccination), one of which was a known anti-PfRH5 clonotype: `IGHV1-18.IGHJ5.CDR3_len16` (Fig. 7).

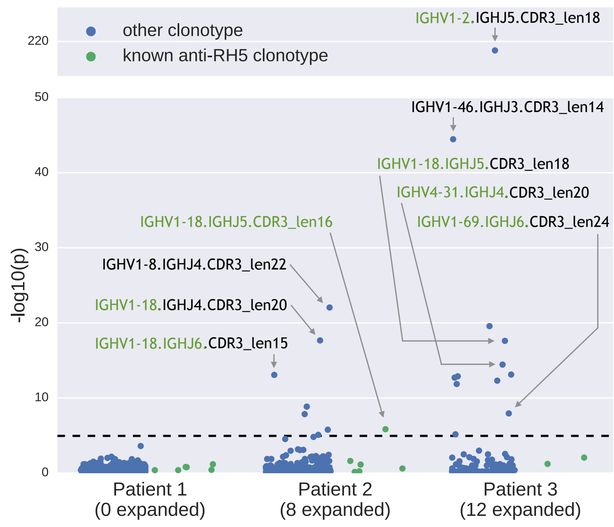


Figure 7: Strip plot showing p-value (two-proportion z-test) for increase in clonotype proportion. Colored sections in labelled clonotype names indicate correspondence to known anti-PfRH5 V-J gene segments or clonotypes. Dashed line shows p-value threshold for familywise error rate = 0.05 (Holm method).

### Individuals had disparate responses to vaccination

- Patient memory repertoires responded very differently to the vaccine candidate (Fig. 8). Patient 1 showed relatively little change in the structure of their repertoire (no expanded clonotypes). There was no overlap between expanded clonotypes in the repertoires of Patient 2 (eight expanded) and Patient 3 (twelve expanded).

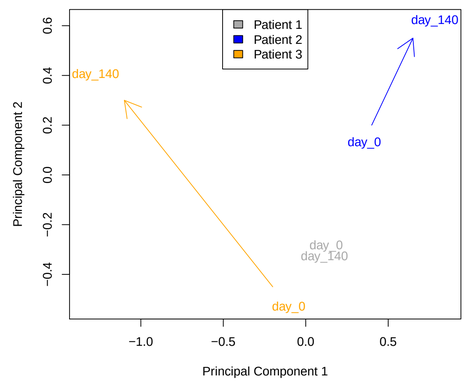


Figure 8: MDS plot of normalised clonotype frequencies in the memory reportoire shows different trajectories of response between patients.

- The composition of the memory repertoire subset consisting of known anti-PfRH5 clonotypes is dissimilar between individuals (Fig. 9).

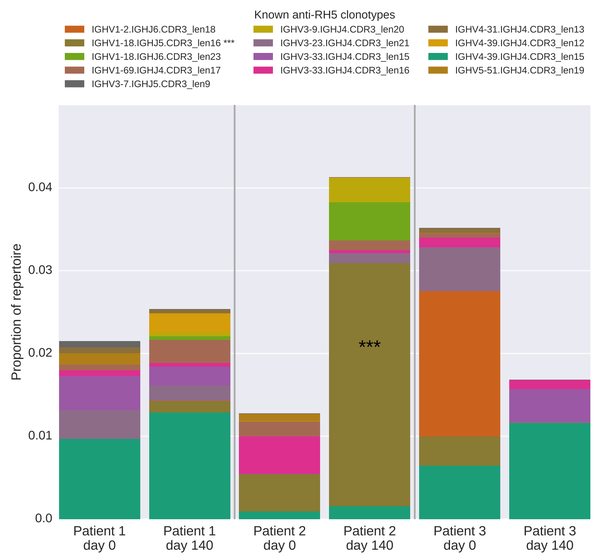


Figure 9: Proportions of memory repertoires occupied by clonotypes of known anti-PfRH5 monoclonal antibodies. *** indicates significantly-expanded `IGHV1-18.IGHJ5.CDR3_len16` clonotype in Patient 2, from 0.46% to 2.94% (adj. $p = 6.97 \times 10^{-3}$).

### High mutational frequency of expanded clonotypes suggests activation

- Activated B-cells can undergo somatic hypermutation, with selection for increased antigen-Ig binding affinity.
- An increase in the mutational frequencies of BCR clones with expanded clonotypes versus non-expanded clonotypes suggests this affinity maturation has occurred in response to vaccination (Fig. 10).

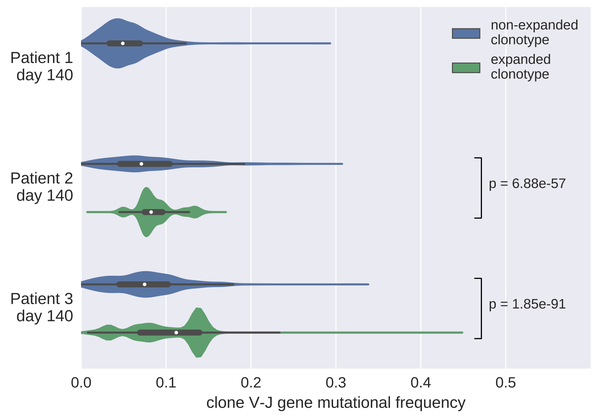


Figure 10: Mutational frequency (proportion of mutated bases) in the IGH V and J gene segments of BCR sequences in the day 140 memory repertoires. Significant increases in mutational frequency for expanded clonotypes (Mann-Whitney U test) in Patient 2 and Patient 3 are indicated.

### Induced memory response was predominantly IgA

- Naive B-cells produce IgH sequences with IgM and/or IgD isotype constant domains. After activation, class switching allows production of antibodies of other isotypes with different effector functions.
- The proportion of IgA isotype clones increases after vaccination, due to increase among expanded clonotypes (Fig. 11). Prevalant IgA1/A2 isotypes suggest a mucosal immune response, likely to be vaccine-induced.

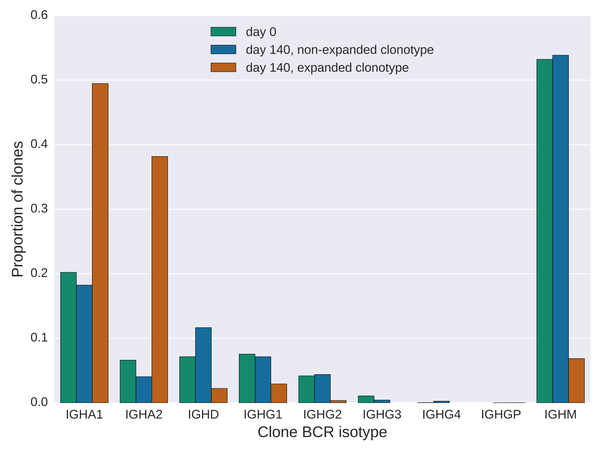


Figure 11: Proportions of isotypes across all patients in the memory repertoire at day 0 among all clones, at day 140 among non-expanded clones and at day 140 among expanded clones.

### Future directions

- Integrating data from earlier timepoints e.g. first appearance of interesting clonotypes after prime/boost, clonotype reuse after boosting, overlaps with the Ig-secreting plasmablast compartment.
- Comparison to data from a Duffy-based *Plasmodium vivax* malaria vaccine candidate, which uses a recombinant protein delivery system.
- Cloning and expression of Igs with expanded clonotypes, followed by validation of PfRH5 affinity/inhibition in binding assays.

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